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(71) Applicant: MILLENNIUM BIOTHERAPEUTICS, INC. [US/US]; 620 Memorial Drive, Cambridge, MA 02142 (US).			
(72) Inventors: HOLTZMAN, Douglas, A.; 821 Centre Street #6, Jamaica Plain, MA 02130 (US). GOODEARL, Andrew, D., J.; 13 Indian Ridge Road, Natick, MA 01760 (US).			
(74) Agent: MEIKLEJOHN, Anita, L.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).			
(54) Title: TANGO-71, TANGO-73, TANGO-74, TANGO-76, AND TANGO-83 NUCLEIC ACID MOLECULES AND POLYPEPTIDES			
(57) Abstract			
<p>The invention relates to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83, and uses thereof.</p>			

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TANGO-71, TANGO-73, TANGO-74, TANGO-76, AND TANGO-83
NUCLEIC ACID MOLECULES AND POLYPEPTIDES

Cross Reference to Related Applications

This application claims priority from U.S. Serial
5 Number 60/054,966, filed August 6, 1997 and U.S. Serial
Number 60/058,108, filed September 5, 1997.

Summary of the Invention

The invention relates to the discovery and characterization of Tango-71, Tango-73, Tango-74, Tango-10 76, and Tango-83. Tango-71 is a human protein which is approximately 90% identical to murine ADAMTS-1. Tango-73 is a human protein that is 48% identical to rat RVP.1 (Briehl et al., *Mol. Endocrinol.* 5:1381, 1991). Tango-74 is a human protein with homology to TRAIL receptor (Pan 15 et al., *Science* 276:111, 1997). Tango-76 is a rat protein which is approximately 40% identical to murine ADAMTS-1. Tango-83 is expressed by stimulated human astrocytes.

The invention features isolated nucleic acid 20 molecules encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides; isolated nucleic acid molecules encoding polypeptides which are substantially similar to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; and isolated nucleic acid molecules which 25 hybridize under stringent conditions to a nucleic acid molecule having the sequence of the protein coding portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9.

The invention also features a host cell which 30 includes an isolated nucleic acid molecule encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 and a nucleic acid vector (e.g., an expression vector; a vector which includes a regulatory element; a vector which includes a regulatory element selected from the group 35 consisting of the cytomegalovirus hCMV immediate early

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gene, the early promoter of SV40 adenovirus, the late promoter of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors; vector which includes a regulatory element which directs tissue-specific expression; a vector which 10 includes a reporter gene; a vector which includes a reporter gene selected from the group selected from the group consisting of β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, G418^r), 15 dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT); a vector that is a plasmid, a vector that is a virus; and a vector that 20 is a retrovirus) containing an isolated nucleic acid molecule encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention also features substantially pure Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 25 polypeptides; a substantially pure polypeptide which includes a first portion and a second portion, the first portion including a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide and the second portion including a detectable marker.

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The invention also features an antibody that selectively binds to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., a monoclonal antibody).

5 The invention also features a pharmaceutical composition which includes a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide.

The invention includes methods for diagnosing a disorder associated with aberrant expression of a protein 10 of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83), the method including obtaining a biological sample from a patient and measuring the expression of the protein in the biological sample, wherein increased or decreased expression of the protein 15 in the biological sample compared to a control indicates that the patient suffers from a disorder associated with aberrant expression of the protein.

The invention encompasses isolated nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-20 76, or Tango-83 or a polypeptide fragment thereof; vectors containing these nucleic acid molecules; cells harboring recombinant DNA encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; fusion proteins which include all or a portion of Tango-71, Tango-73, Tango-74, 25 Tango-76, or Tango-83; transgenic animals which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; and recombinant knock-out animals which fail to express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention encompasses nucleic acids that have 30 a sequence that is substantially identical to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 nucleic acid sequence. A nucleic acid molecule which is substantially identical to a given reference nucleic acid molecule is hereby defined as a nucleic acid molecule having a 35 sequence that has at least 85%, preferably 90%, and more

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preferably 95%, 98%, 99% or more identity to the sequence of the given reference nucleic acid molecule.

The invention also includes polypeptides which are substantially identical to Tango-71, Tango-73, Tango-74, 5 Tango-76, or Tango-83 (e.g., polypeptides that are substantially identical to the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10).

A polypeptide which is "substantially identical" 10 to a given reference polypeptide molecule is a polypeptide having an amino acid sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the amino acid sequence of the given reference polypeptide.

15 To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second 20 amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in 25 the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., 30 overlapping positions) x 100). Preferably, the two sequences are the same length.

The determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a 35 mathematical algorithm utilized for the comparison of two

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sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the 5 NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 10 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein molecules of the invention. To obtain 15 gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules. Id. 20 When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for 25 the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid 30 sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating 35 percent identity, only exact matches are counted.

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The nucleic acid molecules of the invention can be inserted into vectors, as described below, which will facilitate expression of the insert. The nucleic acid molecules and the polypeptides they encode can be used 5 directly as diagnostic or therapeutic agents, or (in the case of a polypeptide) can be used to generate antibodies that, in turn, are therapeutically useful. Accordingly, expression vectors containing the nucleic acid molecules of the invention, cells transfected with these vectors, 10 the polypeptides expressed, and antibodies generated, against either the entire polypeptide or an antigenic fragment thereof, are among the preferred embodiments.

A transformed cell is any cell into which (or into an ancestor of which) has been introduced, by means of 15 recombinant DNA techniques, a nucleic acid encoding a polypeptide of the invention (e.g., a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide).

An isolated nucleic acid molecule is a nucleic acid molecule that is separated from the 5' and 3' coding 20 sequences with which it is immediately contiguous in the naturally occurring genome of an organism. Isolated nucleic acid molecules include nucleic acid molecule which are not naturally occurring, e.g., nucleic acid molecules created by recombinant DNA techniques.

25 Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand.

30 The invention also encompasses nucleic acid molecules that hybridize, preferably under stringent conditions, to a nucleic acid molecule encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., a nucleic acid molecule having the sequence shown 35 in SEQ ID NO:1, 3, 5, 7, or 9). Preferably the

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hybridizing nucleic acid molecule consists of 400, more preferably 200 nucleotides. Preferred hybridizing nucleic acid molecules have a biological activity possessed by Tango-71, Tango-73, Tango-74, Tango-76, or 5 Tango-83.

The invention also features substantially pure or isolated Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides, including those that correspond to various functional domains of Tango-71, Tango-73, Tango-10 74, Tango-76, or Tango-83, or fragments thereof.

The polypeptides of the invention can be produced recombinantly, chemically synthesized, or purified from tissues in which they are naturally expressed, according to standard biochemical methods of purification.

15 Also included in the invention are functional polypeptides, which possess one or more of the biological functions or activities of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. These functions include the ability to bind some or all of the proteins which 20 normally bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. A functional polypeptide is also considered within the scope of the invention if it serves as an antigen for production of antibodies that specifically bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-25 83. In many cases, functional polypeptides retain one or more domains present in the naturally-occurring form of the polypeptide.

The functional polypeptides may contain a primary amino acid sequence that has been modified from those 30 disclosed herein. Preferably these modifications consist of conservative amino acid substitutions, as described herein.

The terms "protein" and "polypeptide" are used herein to describe any chain of amino acids, regardless 35 of length or post-translational modification (for

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example, glycosylation or phosphorylation). Thus, the term "Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides" includes full-length, naturally occurring Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 5 protein, as well a recombinantly or synthetically produced polypeptide that correspond to a full-length naturally occurring Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or to particular domains or portions of a naturally occurring protein. The term also 10 encompasses mature Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 which has an added amino-terminal methionine (useful for expression in prokaryotic cells).

The term "purified" as used herein refers to a nucleic acid or peptide that is substantially free of 15 cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

Polypeptides or other compounds of interest are 20 said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of 25 interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The invention also features antibodies, e.g., monoclonal, polyclonal, and engineered antibodies, which 30 specifically bind Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. An antibody which specifically binds to a given antigen is an antibody that recognizes and binds to a particular antigen, but which does not substantially recognize or bind to other molecules in a sample, e.g., a

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biological sample, which includes Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

The invention also features antagonists and agonists of Tango-71, Tango-73, Tango-74, Tango-76, or 5 Tango-83 that inhibit one or more of the biological activities of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Suitable antagonists can include small molecules (i.e., molecules with a molecular weight below about 500), large molecules (i.e., molecules with a 10 molecular weight above about 500), neutralizing antibodies, polypeptides which compete with a native form of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 for binding to a protein, and nucleic acid molecules that interfere with transcription of Tango-71, Tango-73, 15 Tango-74, Tango-76, or Tango-83 (for example, antisense nucleic acid molecules and ribozymes). Agonists of Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 also include small and large molecules, and antibodies other than neutralizing antibodies.

20 The invention also features molecules which can increase or decrease the expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (e.g., by influencing transcription or translation). Small molecules (i.e., molecules with a molecular weight below about 500), large 25 molecules (i.e., molecules with a molecular weight above about 500), and nucleic acid molecules that can be used to inhibit the expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (for example, antisense and ribozyme molecules) or to enhance their expression (for 30 example, molecules that bind to a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcription regulatory sequences and increase Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcription).

In addition, the invention features substantially 35 pure polypeptides that functionally interact with Tango-

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71, Tango-73, Tango-74, Tango-76, or Tango-83, and the nucleic acid molecules that encode them.

The invention encompasses methods for treating disorders associated with aberrant expression or activity 5 of a protein of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83). Thus, the invention includes methods for treating disorders associated with excessive expression or activity of a protein of the invention. Such methods entail administering a compound 10 which decreases the expression or activity of the protein. The invention also includes methods for treating disorders associated with insufficient expression or activity of a protein of the invention. These methods entail administering a compound which 15 increases the expression or activity of the protein.

The invention also features methods for detecting a protein of the invention (i.e., Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83). Such methods include: obtaining a biological sample; contacting the sample with 20 an antibody that specifically binds the protein under conditions which permit specific binding; and detecting any antibody-protein complexes formed.

In addition, the present invention encompasses methods and compositions for the diagnostic evaluation, 25 typing, and prognosis of disorders associated with inappropriate expression or activity of a protein of the invention. For example, the nucleic acid molecules of the invention can be used as diagnostic hybridization probes to detect, for example, inappropriate expression 30 of a protein of the invention or mutations in the gene encoding a protein of the invention gene. Such methods may be used to classify cells by the level of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 expression.

The invention encompasses methods for diagnosing a 35 disorder associated with aberrant activity of a protein

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of the invention, the methods including obtaining a biological sample from a patient and measuring the activity of the protein in the biological sample, wherein increased or decreased activity in the biological sample 5 compared to a control indicates that the patient suffers from a disorder associated with aberrant activity of the protein.

The nucleic acid molecules of the invention can be used as primers for diagnostic PCR analysis for the 10 identification of gene mutations, allelic variations and regulatory defects in the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene. The present invention further provides for diagnostic kits for the practice of such methods.

15 The invention features methods of identifying compounds that modulate the expression or activity of a protein of the invention by assessing the expression or activity of the protein in the presence and absence of a selected compound. A difference in the level of 20 expression or activity of the protein indicates that the selected compound is capable of modulating expression or activity of the protein. Expression can be assessed either at the level of gene expression (e.g., by measuring mRNA) or protein expression by techniques that 25 are well known to skilled artisans. The activity of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be assessed functionally.

The preferred methods and materials are described below in examples which are meant to illustrate, not 30 limit, the invention. Skilled artisans will recognize methods and materials that are similar or equivalent to those described herein, and that can be used in the practice or testing of the present invention.

Unless otherwise defined, all technical and 35 scientific terms used herein have the same meaning as

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commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Brief Description of the Drawings

Figure 1 nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of Tango-71.

Figure 2 Nucleotide acid sequence (SEQ ID NO:3) and deduced amino acid sequence (SEQ ID NO:4) of Tango-73.

Figure 3 Nucleotide acid sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ ID NO:6) of Tango-74. The ATG encoding the first Met is boxed as is the ATC encoding the final Ile.

Figure 4 Nucleotide acid sequence of a 3' non-coding portion of Tango-74 (SEQ ID NO:11).

Figure 5 Alignment of a portion of the amino acid sequence of Tango-74 (SEQ ID NO:6) and the amino acid sequence of TRAIL.

Figure 6 Partial nucleotide sequence (SEQ ID NO:7) and deduced amino acid sequence (SEQ ID NO:8) of Tango-76.

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Figure 7 Nucleotide sequence of a 5' portion of Tango-83 (SEQ ID NO:9).

Figure 8 Nucleotide sequence of a 3' portion of Tango-83 (SEQ ID NO:10).

5 Figure 9 Alignment of amino acid sequence of Tango-71 and the amino acid sequence of ADAMTS-1.

Figure 10 Alignment of the amino acid sequence of Tango-73 and the amino acid sequence of RVPI.

10 Figure 11 Alignment of the amino acid sequence of Tango-73 and TMVCF.

Figure 12 Northern blot analysis of Tango-73 mRNA.

Figure 13 Northern blot analysis of Tango-83 mRNA.

Figure 14 Alignment of amino acid sequence of Tango-76 and ADAMTS-1.

15

Detailed Description

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Nucleic Acid Molecules

The Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 nucleic acid molecules of the invention can be 20 cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (i.e., either a sense or an antisense strand). Fragments of these molecules are also considered within the scope of the invention, and can be produced, for example, by the polymerase chain 25 reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription.

The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that 30 differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. In addition, these nucleic acid molecules are not limited to sequences that only encode

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polypeptides, and thus, can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The nucleic acid molecules of the invention can be 5 synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as the cell of a mammal. Thus, the nucleic acids can be those of a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. Combinations or 10 modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass fragments that are not found as such in the natural state. Thus, the invention 15 encompasses recombinant molecules, such as those in which a nucleic acid molecule (for example, an isolated nucleic acid molecule encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83) is incorporated into a vector (for example, a plasmid or viral vector) or into the genome of 20 a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

In the event the nucleic acid molecules of the 25 invention encode or act as antisense molecules, they can be used for example, to regulate translation of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. Techniques associated with detection or regulation of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 30 expression are well known to skilled artisans and can be used to diagnose and/or treat disorders associated with aberrant Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 expression.

The invention also encompasses nucleic acid 35 molecules that hybridize under stringent conditions to a

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nucleic acid molecule encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide (e.g., nucleic acid molecules having the sequence of the protein coding portion of SEQ ID NO:1, 3, 5, 7, or 9). The cDNA sequences described herein can be used to identify these hybridizing nucleic acids, which include, for example, nucleic acids that encode homologous polypeptides in other species and splice variants of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene in humans or other mammals. Accordingly, the invention features methods of detecting and isolating these nucleic acid molecules. Using these methods, a sample (for example, a nucleic acid library, such as a cDNA or genomic library) is contacted (or "screened") with a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-specific probe (for example, a fragment of SEQ ID NO:1, 3, 5, 7, or 9 that is at least 25 or 50 or 100 nucleotides long). The probe will selectively hybridize to nucleic acids encoding related polypeptides (or to complementary sequences thereof). The probe, which can contain at least 25 (for example, 25, 50, 100, or 200 nucleotides) can be produced using any of several standard methods (see, for example, Ausubel et al., "Current Protocols in Molecular Biology, Vol. I," Green Publishing Associates, Inc., and John Wiley & Sons, Inc., NY, 1989). For example, the probe can be generated using PCR amplification methods in which oligonucleotide primers are used to amplify a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-specific nucleic acid sequence that can be used as a probe to screen a nucleic acid library and thereby detect nucleic acid molecules (within the library) that hybridize to the probe.

One single-stranded nucleic acid is said to hybridize to another if a duplex forms between them. This occurs when one nucleic acid contains a sequence

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that is the reverse and complement of the other (this same arrangement gives rise to the natural interaction between the sense and antisense strands of DNA in the genome and underlies the configuration of the "double helix"). Complete complementarity between the hybridizing regions is not required in order for a duplex to form; it is only necessary that the number of paired bases is sufficient to maintain the duplex under the hybridization conditions used.

10 Typically, hybridization conditions are of low to moderate stringency. These conditions favor specific interactions between completely complementary sequences, but allow some non-specific interaction between less than perfectly matched sequences to occur as well. After 15 hybridization, the nucleic acids can be "washed" under moderate or high conditions of stringency to dissociate duplexes that are bound together by some non-specific interaction (the nucleic acids that form these duplexes are thus not completely complementary).

20 As is known in the art, the optimal conditions for washing are determined empirically, often by gradually increasing the stringency. The parameters that can be changed to affect stringency include, primarily, temperature and salt concentration. In general, the 25 lower the salt concentration and the higher the temperature, the higher the stringency. Washing can be initiated at a low temperature (for example, room temperature) using a solution containing a salt concentration that is equivalent to or lower than that of 30 the hybridization solution. Subsequent washing can be carried out using progressively warmer solutions having the same salt concentration. As alternatives, the salt concentration can be lowered and the temperature maintained in the washing step, or the salt concentration 35 can be lowered and the temperature increased. Additional

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parameters can also be altered. For example, use of a destabilizing agent, such as formamide, alters the stringency conditions.

In reactions where nucleic acids are hybridized, 5 the conditions used to achieve a given level of stringency will vary. There is not one set of conditions, for example, that will allow duplexes to form between all nucleic acids that are 85% identical to one another; hybridization also depends on unique features of 10 each nucleic acid. The length of the sequence, the composition of the sequence (for example, the content of purine-like nucleotides versus the content of pyrimidine-like nucleotides) and the type of nucleic acid (for example, DNA or RNA) affect hybridization. An additional 15 consideration is whether one of the nucleic acids is immobilized (for example, on a filter).

An example of a progression from lower to higher stringency conditions is the following, where the salt content is given as the relative abundance of SSC (a salt 20 solution containing sodium chloride and sodium citrate; 2X SSC is 10-fold more concentrated than 0.2X SSC). Nucleic acids are hybridized at 42°C in 2X SSC/0.1% SDS (sodium dodecylsulfate; a detergent) and then washed in 0.2X SSC/0.1% SDS at room temperature (for conditions of 25 low stringency); 0.2X SSC/0.1% SDS at 42°C (for conditions of moderate stringency); and 0.1X SSC at 68°C (for conditions of high stringency). Washing can be carried out using only one of the conditions given, or each of the conditions can be used (for example, washing 30 for 10-15 minutes each in the order listed above). Any or all of the washes can be repeated. As mentioned above, optimal conditions will vary and can be determined empirically.

A second set of conditions that are considered 35 "stringent conditions" are those in which hybridization

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is carried out at 50°C in Church buffer (7% SDS, 0.5% NaHPO₄, 1 M EDTA, 1% BSA) and washing is carried out at 50°C in 2X SSC.

Once detected, the nucleic acid molecules can be isolated by any of a number of standard techniques (see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual," 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

The invention also encompasses: (a) expression vectors that contain any of the foregoing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83-related coding sequences operatively associated with a regulatory element (examples of which are given below) that directs the expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide, nucleic acid sequences that are unrelated to nucleic acid sequences encoding Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83, such as molecules encoding a reporter or marker; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention in the host cell.

Recombinant nucleic acid molecules can contain a sequence encoding a soluble Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide; mature Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; or Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 having a signal sequence. A full length Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide; a domain of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; or a fragment thereof may be fused to additional polypeptides, as

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described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 or a form that encodes a polypeptide which facilitates secretion. In 5 the latter instance, the polypeptide is typically referred to as a proprotein, which can be converted into an active form by removal of the signal sequence, for example, within the host cell. Proproteins can be converted into the active form of the protein by removal 10 of the inactivating sequence.

The regulatory elements referred to above include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or 15 otherwise regulate gene expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major 20 operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.

25 Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), 30 adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and 35 xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the

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practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include 5 a first portion and a second portion; the first portion being a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide and the second portion being, for example, the reporter described above or an immunoglobulin constant region.

10 The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression 15 vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding 20 Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression 25 vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 nucleotide sequences; or mammalian cell 30 systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for

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example, the adenovirus late promoter and the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the 5 use intended for the gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions containing Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides or for raising 10 antibodies to those polypeptides, vectors that are capable of directing the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 15 *EMBO J.* 2:1791, 1983), in which the coding sequence of the insert may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, *Nucleic Acids Res.* 13:3101-3109, 1985; Van Heeke and 20 Schuster, *J. Biol. Chem.* 264:5503-5509, 1989); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 25 S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

30 In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequence of the insert may be cloned individually into non-essential 35 regions (for example the polyhedrin gene) of the virus

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and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded 5 recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (for example, see Smith et al., *J. Virol.* 46:584, 1983; 10 Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleic acid molecule of the invention may be ligated to an 15 adenovirus transcription/translation control complex, for example, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome 20 (for example, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene product in infected hosts (for example, see Logan and Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659, 25 1984). Specific initiation signals may also be required for efficient translation of inserted nucleic acid molecules. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire gene or cDNA, including its own initiation codon and 30 adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the 35 ATG initiation codon, must be provided. Furthermore, the

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initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of 5 origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:516-544, 1987).

10 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (for example, glycosylation) and processing (for example, 15 cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems 20 can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the 25 gene product may be used. The mammalian cell types listed above are among those that could serve as suitable host cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For 30 example, cell lines which stably express a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 sequences described above may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA 35 controlled by appropriate expression control elements

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(for example, promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 5 1-2 days in an enriched media, and then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can 10 be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that 15 affect the endogenous activity of the gene product.

A number of selection systems can be used. For example, the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska and Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817, 1980) genes can be employed in tk⁻, hgprt⁻ or aprt⁻ cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: 20 dhfr, which confers resistance to methotrexate (Wigler et al., *Proc. Natl. Acad. Sci. USA* 77:3567, 1980; O'Hare et al., *Proc. Natl. Acad. Sci. USA* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, *Proc. Natl. Acad. Sci. USA* 78:2072, 1981); neo, 25 which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., *J. Mol. Biol.* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre et al., *Gene* 30:147, 1984).

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Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 nucleic acid molecules are useful in genetic mapping and chromosome identification.

Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Polypeptides

The Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides described herein are those encoded by any of the nucleic acid molecules described above and include Tango-71, Tango-73, Tango-74, Tango-76, and 10 Tango-83 fragments, mutants, truncated forms, and fusion proteins. These polypeptides can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, for the identification of other cellular gene 15 products or compounds that can modulate the activity or expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83, and as pharmaceutical reagents useful for the treatment of disorders associated with aberrant expression or activity of Tango-71, Tango-73, Tango-74, 20 Tango-76, or Tango-83.

Preferred polypeptides are substantially pure Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides, including those that correspond to the polypeptide with an intact signal sequence, the secreted 25 form of a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide. Especially preferred are polypeptides that are soluble under normal physiological conditions.

The invention also encompasses polypeptides that 30 are functionally equivalent to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83. These polypeptides are equivalent to Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 in that they are capable of carrying out one or more of the functions of Tango-71, Tango-73, Tango-74, 35 Tango-76, and Tango-83 in a biological system. Preferred

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Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides have 20%, 40%, 50%, 75%, 80%, or even 90% of one or more of the biological activities of the full-length, mature human form of Tango-71, Tango-73, Tango-
5 74, Tango-76, and Tango-83. Such comparisons are generally based on an assay of biological activity in which equal concentrations of the polypeptides are used and compared. The comparison can also be based on the amount of the polypeptide required to reach 50% of the
10 maximal stimulation obtainable.

Functionally equivalent proteins can be those, for example, that contain additional or substituted amino acid residues. Substitutions may be made on the basis of similarity in polarity, charge, solubility,
15 hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. Amino acids that are typically considered to provide a conservative substitution for one another are specified in the summary of the invention.

20 Polypeptides that are functionally equivalent to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be made using random mutagenesis techniques well known to those skilled in the art. It is more likely, however, that such polypeptides will be generated by site-directed
25 mutagenesis (again using techniques well known to those skilled in the art). These polypeptides may have increased functionality or decreased functionality.

To design functionally equivalent polypeptides, it is useful to distinguish between conserved positions and
30 variable positions. This can be done by aligning the amino acid sequence of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 with the amino acid sequence of the homologous protein from another species. Skilled artisans will recognize that conserved amino acid
35 residues are more likely to be necessary for preservation

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of function. Thus, it is preferable that conserved residues are not altered.

Mutations within the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 coding sequence can be made to 5 generate variant Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 genes that are better suited for expression in a selected host cell. For example, N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product 10 that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences 15 which occur, and/or an amino acid deletion at the second position of any one or more of such recognition sequences, will prevent glycosylation at the modified tripeptide sequence (see, for example, Miyajima et al., *EMBO J.* 5:1193, 1986).

20 Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell 25 lines (*Proc. Natl. Acad. Sci. USA* 88: 8972-8976, 1991). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues.

30 Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} ·nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

The polypeptides of the invention can be expressed 35 fused to another polypeptide, for example, a marker

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polypeptide or fusion partner. For example, the polypeptide can be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein or a hemagglutinin tag to facilitate purification of 5 protein expressed in eukaryotic cells.

The polypeptides of the invention can be chemically synthesized (for example, see Creighton, "Proteins: Structures and Molecular Principles," W.H. Freeman & Co., NY, 1983), or, perhaps more 10 advantageously, produced by recombinant DNA technology as described herein. For additional guidance, skilled artisans may consult Ausubel et al. (*supra*), Sambrook et al. ("Molecular Cloning, A Laboratory Manual," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989), and, 15 particularly for examples of chemical synthesis Gait, M.J. Ed. ("Oligonucleotide Synthesis," IRL Press, Oxford, 1984).

The invention also features polypeptides that interact with Tango-71, Tango-73, Tango-74, Tango-76, or 20 Tango-83 (and the genes that encode them) and thereby alter the function of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Interacting polypeptides can be identified using methods known to those skilled in the art. One suitable method is the "two-hybrid system," 25 which detects protein interactions *in vivo* (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available from Clontech (Palo Alto, CA).

Transgenic animals

30 Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides can also be expressed in transgenic animals. These animals represent a model system for the study of disorders that are caused by or exacerbated by overexpression or underexpression of Tango-71, Tango-73, 35 Tango-74, Tango-76, or Tango-83, and for the development

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of therapeutic agents that modulate the expression or activity of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

Transgenic animals can be farm animals (pigs, 5 goats, sheep, cows, horses, rabbits, and the like) rodents (such as rats, guinea pigs, and mice), non-human primates (for example, baboons, monkeys, and chimpanzees), and domestic animals (for example, dogs and cats). Transgenic mice are especially preferred.

10 Any technique known in the art can be used to introduce a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (U.S. 15 Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148, 1985); gene targeting into embryonic stem cells (Thompson et al., *Cell* 56:313, 1989); and electroporation of embryos (Lo, *Mol. Cell. Biol.* 3:1803, 20 1983).

The present invention provides for transgenic animals that carry a the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene in all their cells, as well as animals that carry a transgene in some, but not 25 all of their cells. That is, the invention provides for mosaic animals. The transgene can be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene can also be selectively introduced into and activated in a 30 particular cell type (Lasko et al., *Proc. Natl. Acad. Sci. USA* 89:6232, 1992). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

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When it is desired that the Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique 5 is to be used, vectors containing some nucleotide sequences homologous to an endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function 10 of the nucleotide sequence of the endogenous gene. The transgene also can be selectively introduced into a particular cell type, thus inactivating the endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene in only that cell type (Gu et al., *Science* 265:103, 15 1984). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. Once transgenic animals have been generated, expression of the recombinant Tango- 20 71, Tango-73, Tango-74, Tango-76, or Tango-83 gene can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to determine whether integration of the transgene has taken place. The level of mRNA expression 25 of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of Tango-71, Tango-73, 30 Tango-74, Tango-76, or Tango-83 gene-expressing tissue can also be evaluated immunocytochemically using antibodies specific for the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transgene product.

For a review of techniques that can be used to 35 generate and assess transgenic animals, skilled artisans

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can consult Gordon (*Intl. Rev. Cytol.* 115:171-229, 1989), and may obtain additional guidance from, for example: Hogan et al. "Manipulating the Mouse Embryo" (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1986; Krimpenfort et al., *Bio/Technology* 9:86, 1991; Palmiter et al., *Cell* 41:343, 1985; Kraemer et al., "Genetic Manipulation of the Early Mammalian Embryo," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1985; Hammer et al., *Nature* 315:680, 1985; Purcel et al., *Science*, 244:1281, 1986; 5 Wagner et al., U.S. Patent No. 5,175,385; and Krimpenfort et al., U.S. Patent No. 5,175,384 (the latter two publications are hereby incorporated by reference).

Anti-Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Antibodies

15 Human Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides (or immunogenic fragments or analogs) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example, 20 "Solid Phase Peptide Synthesis," *supra*; Ausubel et al., *supra*). In general, the peptides can be coupled to a carrier protein, such as KLH, as described in Ausubel et al., *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies can be purified by peptide 25 antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or polypeptide. Host animals include rabbits, mice, guinea pigs, and rats. 30 Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, 35 polyanions, peptides, oil emulsions, keyhole limpet

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hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are 5 contained in the sera of the immunized animals.

Antibodies within the invention therefore include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab'), fragments, and 10 molecules produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptides described above and standard 15 hybridoma technology (see, for example, Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel et al., *supra*).

20 In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler et al., *Nature* 256:495, 1975, and U.S. Patent No. 4,376,110; the human 25 B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983), and the EBV-hybridoma technique (Cole et al., "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be 30 of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this a particularly useful method of 35 production.

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Once produced, polyclonal or monoclonal antibodies are tested for specific Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 recognition by Western blot or immunoprecipitation analysis by standard methods, e.g., 5 as described in Ausubel et al., *supra*. Antibodies that specifically recognize and bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 are useful. For example, such antibodies can be used in an immunoassay to monitor the level of Tango-71, Tango-73, Tango-74, Tango-76, or 10 Tango-83 produced by a mammal (for example, to determine the amount or subcellular location of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83).

Preferably, antibodies of the invention are produced using fragments of the Tango-71, Tango-73, 15 Tango-74, Tango-76, or Tango-83 protein which lie outside highly conserved regions and appear likely to be antigenic, by criteria such as high frequency of charged residues. In one specific example, such fragments are generated by standard techniques of PCR, and are then 20 cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., *supra*.

In some cases it may be desirable to minimize the 25 potential problems of low affinity or specificity of antisera. In such circumstances, two or three fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, preferably including at 30 least three booster injections.

Antisera is also checked for its ability to immunoprecipitate recombinant Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 protein or control proteins, such as glucocorticoid receptor, CAT, or luciferase.

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The antibodies can be used, for example, in the detection of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 in a biological sample as part of a diagnostic assay. Antibodies also can be used in a 5 screening assay to measure the effect of a candidate compound on expression or localization of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Additionally, antibodies can be used in conjunction with the gene 10 therapy techniques described to, for example, evaluate the normal and/or engineered Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-expressing cells prior to their introduction into the patient. Such antibodies 15 additionally can be used in a method for inhibiting abnormal Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 activity.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851, 1984; Neuberger et al., *Nature*, 312:604, 1984; Takeda et al., *Nature*, 20 314:452, 1984) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are 25 derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 30 4,946,778, 4,946,778, and 4,704,692) can be adapted to produce single chain antibodies against a Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino 35 acid bridge, resulting in a single chain polypeptide.

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Antibody fragments that recognize and bind to specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to F(ab')₂ fragments that can be produced by pepsin 5 digestion of the antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science*, 246:1275, 1989) to allow rapid and easy identification of 10 monoclonal Fab fragments with the desired specificity.

Antibodies to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can, in turn, be used to generate anti-idiotype antibodies that resemble a portion of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 using 15 techniques well known to those skilled in the art (see, e.g., Greenspan et al., *FASEB J.* 7:437, 1993; Nissinoff, *J. Immunol.* 147:2429, 1991). For example, antibodies that bind to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 and competitively inhibit the binding of a 20 binding partner of the protein can be used to generate anti-idiotypes that resemble a binding partner binding domain of the protein and, therefore, bind and neutralize a binding partner of the protein. Such neutralizing anti-idiotypic antibodies or Fab fragments of such anti- 25 idiotypic antibodies can be used in therapeutic regimens.

Antibodies can be humanized by methods known in the art. For example, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). 30 Fully human antibodies, such as those expressed in transgenic animals are also features of the invention (Green et al., *Nature Genetics* 7:13-21, 1994; see also U.S. Patents 5,545,806 and 5,569,825, both of which are hereby incorporated by reference).

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The methods described herein in which anti-Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 antibodies are employed may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one 5 specific Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 antibody reagent described herein, which may be conveniently used, for example, in clinical settings, to diagnose patients exhibiting symptoms of the disorders described below.

10 Antisense Nucleic Acids

Treatment regimes based on an "antisense" approach involve the design of oligonucleotides (either DNA or RNA) that are complementary to Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. These oligonucleotides 15 bind to the complementary Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a 20 sequence having sufficient complementarily to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may be tested, or triplex formation may be assayed. The ability to hybridize will 25 depend on both the degree of complementarily and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One 30 skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence 35 up to and including the AUG initiation codon, should work

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most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, *Nature* 5 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of the mRNA, could be used in an antisense approach to inhibit translation of endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA. Oligonucleotides 10 complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the 15 invention. Whether designed to hybridize to the 5', 3', or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the 20 oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to 25 quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that 30 these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is 35 preferred that the control oligonucleotide is of

approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (as described, e.g., in Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol et al., *BioTechniques* 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.* 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine,

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1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,
2-methyladenine, 2-methylguanine, 3-methylcytosine,
5-methylcytosine, N6-adenine, 7-methylguanine,
5-methylaminomethyluracil, 5-methoxyaminomethyl-
5 2-thiouracil, beta-D-mannosylqueosine,
5'-methoxycarboxymethyluracil, 5-methoxyuracil,
2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic
acid (v), wybutoxosine, pseudouracil, queosine,
2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-
10 thiouracil, 5-methyluracil, uracil-5-oxyacetic acid
methylester, uracil-5-oxyacetic acid (v), 5-methyl-
2-thiouracil, 2-(3-amino-3-N-2-carboxypropyl) uracil,
(acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at
15 least one modified sugar moiety selected from the group
including, but not limited to, arabinose,
2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense
oligonucleotide comprises at least one modified phosphate
20 backbone selected from the group consisting of a
phosphorothioate, a phosphorodithioate, a
phosphoramidothioate, a phosphoramidate, a
phosphordiamidate, a methylphosphonate, an alkyl
phosphotriester, and a formacetal, or an analog of any of
25 these backbones.

In yet another embodiment, the antisense
oligonucleotide is an α -anomeric oligonucleotide. An
 α -anomeric oligonucleotide forms specific double-stranded
hybrids with complementary RNA in which, contrary to the
30 usual β -units, the strands run parallel to each other
(Gautier et al., *Nucl. Acids. Res.* 15:6625, 1987). The
oligonucleotide is a 2'-O-methylribonucleotide (Inoue
et al., *Nucl. Acids Res.* 15:6131, 1987), or a chimeric
RNA-DNA analog (Inoue et al., *FEBS Lett.* 215:327, 1987).

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Antisense oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (*Nucl. Acids Res.* 16:3209, 1988), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 10 *Proc. Natl. Acad. Sci. USA* 85:7448, 1988).

The antisense molecules should be delivered to cells that express the protein of interest *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules 15 can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells (e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered 20 systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense molecule sufficient to suppress translation of endogenous mRNAs. Therefore, a preferred approach uses a recombinant DNA 25 construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded 30 RNAs that will form complementary base pairs with the endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 transcripts and thereby prevent translation. For example, a vector can be introduced *in vivo* such that 35 it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or

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become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be 5 plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible 10 or constitutive. Such promoters include, but are not limited to: the SV40 early promoter region (Berkoist et al., *Nature* 290:304, 1981); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797, 1988); the herpes 15 thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 78:1441, 1981); or the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 296:39, 1988).

Ribozymes

20 Ribozyme molecules designed to catalytically cleave Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNA transcripts also can be used to prevent translation and expression of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (see, e.g., PCT Publication 25 WO 90/11364; Saraver et al., *Science* 247:1222, 1990). While various ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead 30 ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes 35 is well known in the art (Haseloff et al., *Nature*

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334:585, 1988). There are numerous examples of potential hammerhead ribozyme cleavage sites within the nucleotide sequence of human Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 cDNA. Preferably, the ribozyme is
5 engineered so that the cleavage recognition site is located near the 5' end of the mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also
10 include RNA endoribonucleases (hereinafter "Cech-type ribozymes"), such as the one that occurs naturally in *Tetrahymena Thermophila* (known as the IVS or L-19 IVS RNA), and which has been extensively described by Cech and his collaborators (Zaug et al., *Science* 224:574, 15 1984; Zaug et al., *Science*, 231:470, 1986; Zug et al., *Nature* 324:429, 1986; PCT Application No. WO 88/04300; and Been et al., *Cell* 47:207, 1986). The Cech-type ribozymes have an eight base-pair sequence that hybridizes to a target RNA sequence, whereafter cleavage
20 of the target RNA takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences present in Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83.

As in the antisense approach, the ribozymes can be
25 composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.), and should be delivered to cells which express Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme
30 under the control of a strong constitutive *pol* III or *pol* II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a

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lower intracellular concentration is required for efficiency.

Other Methods for Reducing Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 Expression

5 Endogenous Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 gene expression can also be reduced by inactivating the endogenous gene or its promoter using targeted homologous recombination (see, e.g., U.S. Patent No. 5,464,764). For example, a mutant, non-functional
10 Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions) can be used, with or without a selectable marker and/or a negative selectable
15 marker, to transfect cells that express the endogenous gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene. Such approaches are particularly
20 suited for use in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83. However, this approach can be adapted for use in humans, provided
25 the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 gene expression can be
30 reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (i.e., the promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells in the body (*Helene Anticancer Drug Res.*

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6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.* 660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

Detecting Proteins Associated with Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83

5 The invention also features polypeptides which interact with Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Any method suitable for detecting protein-protein interactions may be employed for identifying transmembrane proteins, intracellular, or extracellular 10 proteins that interact with Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. Among the traditional methods which may be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns of cell lysates or proteins 15 obtained from cell lysates and the use of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 to identify proteins in the lysate that interact with Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83. For these assays, the Tango-71, Tango-73, Tango-74, Tango-76, or 20 Tango-83 polypeptide can be: a full length Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; a soluble extracellular domain of Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83; or some other suitable Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide. 25 Once isolated, such an interacting protein can be identified and cloned and then used, in conjunction with standard techniques, to identify proteins with which it interacts. For example, at least a portion of the amino acid sequence of a protein which interacts with the 30 Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. The amino acid sequence obtained may be used as a guide for the generation of oligonucleotide mixtures 35 that can be used to screen for gene sequences encoding

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the interacting protein. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known (Ausubel, *supra*; and 5 "PCR Protocols: A Guide to Methods and Applications," Innis et al., eds. Academic Press, Inc., NY, 1990).

Additionally, methods may be employed which result directly in the identification of genes which encode proteins which interact with Tango-71, Tango-73, Tango-10 74, Tango-76, or Tango-83. These methods include, for example, screening expression libraries, in a manner similar to the well known technique of antibody probing of λ gt11 libraries, using labeled Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 polypeptide or a Tango-15 71, Tango-73, Tango-74, Tango-76, and Tango-83 fusion protein, e.g., a Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptide or domain fused to a marker such as an enzyme, fluorescent dye, a luminescent protein, or to an IgFc domain.

20 There are also methods which are capable of detecting protein interaction. A method which detects protein interactions *in vivo* is the two-hybrid system (Chien et al., *Proc. Natl. Acad. Sci. USA*, 88:9578, 1991). A kit for practicing this method is available 25 from Clontech (Palo Alto, CA).

Compounds which bind Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83

Compounds which bind Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 can be identified using any 30 standard binding assay. For example, candidate compounds can be bound to a solid support. Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83 is then exposed to the immobilized compound and binding is measured (European Patent Application 84/03564).

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Tango-71 cDNA (Fig. 1; SEQ ID NO:1) was isolated from human melanocytes as follows.

Human melanocytes (Clonetics Corporation; San Diego, CA) were expanded in culture with Melanocyte 5 Growth Media (MGM; Clonetics) according to the recommendations of the supplier. When the cells reached -80-90% confluence, they were starved in MGM without growth factors for 46 hours. The starved cells were then stimulated with complete MGM supplemented with 20 ng/ml 10 TNF (Gibco BRL; Gaithersburg, MD) and cycloheximide (CHI; 40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen; Chatsworth, CA), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

15 Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL). Complementary DNA was directionally cloned into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to 20 construct a plasmid library. Transformants were picked and grown up for single-pass sequencing. Additionally, astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library.

25 Northern blot analysis of Tango-71 expression was performed using Tango-71 labeled with ³²P-dCTP using the Prime-It kit (Stratagene, LaJolla, CA). Human mRNA blots (MTNI and MTNII; Clonetech; Palo Alto, CA) were probed and washed at high stringency as recommended by the 30 manufacturer. Tango-71 is expressed as an approximately 6.0 kb transcript in all tissues: heart brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testes, ovary, small intestine, colon, PBLs.

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The amino acid sequence of a portion of Tango-71 is 90% identical to the amino acid sequence of murine ADAMTS-1 (FIG. 9), a cellular disintegrin and metalloprotease that is thought to be involved in 5 inflammation and development of cancer cachexia (Kuno et al., *J. Biol. Chem.* 272:556, 1997). Based on sequence comparison to ADAMTS-1, Tango-71, using the amino acid numbering in Figure 9, has the following domains: amino acids 1-160 (metalloproteinase domain, partial); amino 10 acids 170-242 (disintegrin domain); amino acids 257-307 (thrombospondin domain). A less apparent thrombospondin domain is present at amino acid 558-608. Portions of Tango-71 shown in Figure 1, but not in Figure 9, may also be homologous ADAMTS-1. Tango-71 may represent the human 15 homolog of ADAMTS-1 or a splice variant thereof.

Tango-71 expression may be androgen regulated. Tango-71 expression in LNCaP cells, an androgen-dependent prostate cancer cell line, is induced by R1881, a testosterone analog. Tango-71 expression is 20 downregulated in LNCaP cells treated with casodex, an anti-androgen.

Tango-73 cDNA (Fig. 2; SEQ ID NO:2) was isolated from human prostate epithelial cells as follows.

Human prostate epithelial cells (Clonetics) were 25 expanded in culture with Prostate Epithelial Growth Medium (PEGM) (Clonetics). When cells reached confluence cells were grown in Prostate Basal Media (Clonetics) for 24 hours. They were stimulated with PEGM (prostate epithelial growth medium; Clonetics) and 40 ug/ml 30 cycloheximide for 3 hours.

Total RNA was isolated using the RNeasy Midi Kit (Qiagen). Poly (A)+ was isolated using the Oligotex beads (Qiagen). Next, cDNA was constructed using the Superscript cDNA Synthesis Kit (Gibco BRL). The cDNA was 35 cloned into the expression vector pMET7 using the SalI

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and NotI sites in the polylinker. Transformants were picked and sequenced.

Northern blot analysis of Tango-73 expression was carried out as described above. This analysis revealed 5 the presence of 4.0 kb and 3.0 kb transcripts in the placenta and liver. A 4.0 kb transcript was present in lung, kidney, thymus, prostate, spleen, testes, and colon, with the highest expression in lung, pancreas, prostate, and testes.

10 The amino acid sequence of Tango-73 is 48% identical to rat RVP.1 (Briechl et al., *Mol. Endocrinol.* 5:1381, 1991) and 46.1% identical to TMVCF (Sirotkin et al., *Genomics* 42:245, 1997).

15 RVP.1 is up-regulated during apoptosis (Briechl et al., *supra*). TMVCF, a 219 amino acid protein with two putative membrane spanning domains, is deleted in velo-cardio-facial syndrome (Sirotkin et al., *supra*).

Tango-83 (Figs. 7 and 8) and Tango-74 cDNAs (Fig. 5) were isolated from human astrocytes as follows.

20 Human astrocytes (Clonetics) were expanded in culture with Astrocyte Growth Media (AGM; Clonetics) according to the recommendations of the supplier. When the cells reached ~80-90% confluence, they were stimulated with 200 units/ml Interleukin 1-Beta 25 (Boehringer Mannheim) and cycloheximide (CHI: 40 micrograms/ml) for 4 hours. Total RNA was isolated using the RNeasy Midi Kit (Qiagen), and the poly A+ fraction was further purified using Oligotex beads (Qiagen).

30 Three micrograms of poly A+ RNA were used to synthesize a cDNA library using the Superscript cDNA Synthesis kit (Gibco BRL). Complementary DNA was directionally cloned into the expression plasmid pMET7 using the SalI and NotI sites in the polylinker to construct a plasmid library. Transformants were picked 35 and grown up for single-pass sequencing. Additionally,

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astrocyte cDNA was ligated into the SalI/NotI sites of the ZipLox vector (Gibco BRL) for construction of a lambda phage cDNA library.

5 Northern blot analysis of Tango-83 expression, performed as described above, revealed that Tango-83 is expressed as an approximately 9.0 kb transcript in brain (FIG. 13).

10 Northern blot analysis, performed as described above, revealed that Tango-74 is expressed as an approximately 4.0 kb transcript in heart, brain, lung, liver, kidney, pancreas, spleen, prostate, testes, ovary, small intestine, colon and peripheral blood lymphocytes. Higher expression was seen in lung, liver, skeletal muscle, spleen, testes, colon and peripheral blood 15 lymphocytes.

The amino acid sequence of Tango-74 is homologous to the amino acid sequence of the TRAIL receptor (Pan et al., *Science* 276:111, 1997) (FIG. 5).

20 Tango-76 cDNA (SEQ ID NO:7) was isolated from an adult rat frontal cortex library. The amino acid sequence of Tango-76 is homologous to the amino acid sequence of ADAMTS-1 (FIG. 14).

25 Northern blot analysis of human mRNA probed with a Tango-76 probe revealed a 4.2 kb band in lung. Analysis of rat mRNA revealed a weak 3.8 kb transcript in heart, brain, spleen, liver, skeletal muscle, and kidney and a weak 1.8 kb transcript in spleen and liver.

Effective Dose

30 Toxicity and therapeutic efficacy of the polypeptides of the invention and the compounds that modulate their expression or activity can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ 35 (the dose therapeutically effective in 50% of the

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population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . Polypeptides or other compounds that exhibit large therapeutic indices are 5 preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

10 The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no 15 toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. 20 A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (that is, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used 25 to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Formulations and Use

Pharmaceutical compositions for use in accordance 30 with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for 35 administration by inhalation or insufflation (either

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through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets 5 or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose 10 or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets may be coated by methods well known in the 15 art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by 20 conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond 25 oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for 30 oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

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For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with 5 the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to 10 deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral 15 administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms 20 as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder 25 form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, for example, containing conventional suppository bases such as cocoa butter or other glycerides.

30 In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, 35 for example, the compounds may be formulated with

suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

5 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be
10 accompanied by instructions for administration.

The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients which can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The nucleic acids, polypeptides, antibodies, or
20 modulatory compounds of the invention can be administered by any standard route of administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, 25 intracisternal, intraperitoneal, transmucosal, or oral. The modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be
30 made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that the preferred route of administration will be intravenous.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - a) a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof;
 - 10 b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof;
 - 15 c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____;
 - 20 d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or the polypeptide encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____; and
 - 25 e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the nucleic

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acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions.

5 2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of:

- a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, the cDNA insert of the 10 plasmid deposited with ATCC as Accession Number _____, or a complement thereof; and
- b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid 15 deposited with ATCC as Accession Number _____.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further 20 comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian 25 host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

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8. An isolated polypeptide selected from the group consisting of:

- a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8;
- b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions; and
- c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

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12. A method for producing a polypeptide selected from the group consisting of:

- a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____;
- b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____; and
- c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, or a complement thereof under stringent conditions;
comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

30 13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising:

- a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and

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b) determining whether the compound binds to the polypeptide in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

5 15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising 10 the steps of:

- a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and
- b) determining whether the nucleic acid probe or 15 primer binds to a nucleic acid molecule in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively 20 hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of:

- 25 a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and
- b) determining whether the polypeptide binds to the test compound.

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20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detecting of test compound/polypeptide binding;
- b) detection of binding using a competition binding assay;
- c) detection of binding using an assay for Tango-71, Tango-73, Tango-74, Tango-76, or Tango-83-mediated signal transduction.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising:

- 20 a) contacting a polypeptide of claim 8 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

FIGURE 1 ($1_{S=1}$)

T	L	P	S	V	A	A	R	L	Y	K	H	P	S	I	R	N	S	V	S	303	
ACG	TTC	TTT	TCG	GTC	GCA	GCC	AGA	TTC	TAC	AAA	CAC	CCC	AGC	ATT	CCT	AAT	TCA	GTT	AGC	1368	
L	V	V	V	K	I	L	V	I	H	D	E	Q	K	G	P	E	V	T	S	323	
CTG	GTC	GTC	GTC	AAG	ATC	TTG	GTC	ATC	CAC	GAT	GAA	CAG	AAG	GGG	CGG	GAA	GTC	ACC	TCC	1428	
N	A	A	L	T	L	R	N	F	C	N	W	Q	K	Q	H	N	P	P	S	343	
AAT	GCT	GCC	CTC	ACT	CTG	CGG	AAC	TTT	TGC	AAC	TGG	CAG	ARG	CAG	CAC	AAC	CCA	CCC	AGT	1488	
D	R	D	A	E	H	Y	D	T	A	I	L	F	T	R	Q	D	L	C	G	363	
GAC	CGG	GAT	GCA	GAG	CAC	TAT	GAC	ACA	GCA	ATT	CTT	TTC	ACC	AGA	CAG	GAC	TTG	TGT	GGG	1548	
S	Q	T	C	D	T	L	G	M	A	D	V	G	T	V	C	D	P	S	R	383	
TCC	CAG	ACA	TGT	GAT	ACT	CTT	GGG	ATG	GCT	GAT	GTT	GGG	ACT	GTC	TGT	GAT	GGG	AGC	AGA	1608	
S	C	S	V	I	E	D	D	G	L	Q	A	A	F	T	T	A	H	E	L	403	
AGC	TCC	TCC	GTC	ATA	GAA	GAT	GAT	GGT	TTA	CAA	GCT	GCC	TTC	ACC	ACA	GCC	CAT	GAA	TTA	1668	
G	H	V	P	N	M	P	H	D	D	A	K	Q	C	A	S	L	N	G	V	423	
GGC	CAC	GTC	TTT	AAC	ATG	CCA	CAT	GAT	GAT	GCA	AAG	CAG	TGT	GCC	AGC	CTT	AAT	GGT	GTC	1728	
N	Q	D	S	H	M	M	A	S	M	L	S	N	L	D	H	S	Q	P	W	443	
AAC	CAG	GAT	TCC	CAC	ATG	ATG	GGG	TCA	ATG	CTT	TCC	AAC	CTG	GAC	CAC	ACC	CAG	CCT	TGG	1788	
S	P	C	S	A	Y	M	I	T	S	F	L	D	N	G	H	G	E	C	L	463	
TCT	CCT	TCC	AGT	GCC	TAC	ATG	ATT	ACA	TCA	TTT	CTG	GAT	AAT	GGT	CAT	GGG	GAA	TGT	TTG	1848	
M	D	K	P	Q	N	P	I	Q	L	P	G	D	L	P	G	T	S	Y	D	483	
ATG	GAC	AAG	CCT	CAG	AAT	CCC	ATA	CAG	CTC	CCA	GGC	GAT	CTC	CCT	GCC	ACC	TCG	TAC	GAT	1908	
A	N	R	Q	C	Q	F	T	F	G	E	D	S	K	H	C	P	D	A	A	503	
GCC	AAC	CGG	CAG	TCC	CAG	TTT	ACA	TTT	GGG	GAG	GAC	TCC	AAA	CAC	TGC	CCC	GAT	GCA	GCC	1968	
S	T	C	S	T	L	W	C	T	G	T	S	G	G	V	L	U	C	Q	T	523	
AGC	ACA	TGT	AGC	ACC	TTG	TGG	TGT	ACC	GGC	ACC	TCT	GGT	GGG	GTC	GTC	GTC	GTC	TGT	CAA	ACC	2028
R	H	F	P	W	A	D	G	T	S	C	G	E	G	K	W	C	I	N	G	543	
AAA	CAC	TTC	CGG	TGG	GGG	GAT	GGC	ACC	AGC	TGT	GCA	GAA	GGG	AAA	TGG	TGT	ATC	AAC	GGC	2088	
R	C	V	N	K	T	D	R	K	H	F	D	T	P	F	H	G	S	W	G	563	
AAG	TGT	GTC	AAC	ACC	GAC	AGA	AGG	CAT	TTT	GAT	ACC	CCT	TTT	CAT	GGA	AGC	TGG	GGA	2148		
M	W	G	P	W	G	D	C	S	R	T	C	G	G	G	V	Q	Y	T	H	583	
ATG	TGG	GGG	CCT	TTG	GGA	GAC	TGT	TCG	AGA	AGC	TCC	GGT	GGA	GGA	GTC	CAG	TAC	AGC	ATG	2208	
R	E	C	D	N	P	V	P	R	N	G	G	K	Y	C	E	G	K	R	V	603	
AGG	GAA	TGT	GAC	AAC	CCA	GTC	CCA	AAG	ATT	GGG	GGG	AAG	TAC	TGT	GAA	GCC	AAA	CGA	GTC	2268	
R	Y	R	S	C	N	L	E	D	C	P	D	N	N	G	K	T	F	R	E	623	
CGC	TAC	AGA	TCC	TGT	AAC	CTT	GAG	GAC	TGT	CCA	GAC	AAT	AAT	GGG	AAA	ACC	TTT	AGA	GAG	2328	
E	Q	C	E	A	H	N	E	F	S	K	A	S	F	G	S	G	P	A	V	643	
GAA	CAA	TGT	GAA	GCA	CAC	AAC	GAG	TTT	TCA	AAA	GCT	TCC	TTT	GGG	AGT	GGG	CCT	GGG	GTC	2388	
E	W	I	P	K	Y	A	G	V	S	P	X	D	R	C	X	L	I	C	Q	663	
GAA	TGG	ATT	CCC	AAG	TAC	GCT	GGC	GTC	TCA	CCA	AAG	GAC	AGG	TGC	AAG	CTC	ATC	TGC	CAA	2448	
A	K	G	I	G	Y	F	F	V	L	Q	P	K	V	V	D	G	T	P	C	683	

GCC ARA GGC ATT GGC TAC TIC TIC GTT TTG CAG CCC AAG GTT GTC GAT GGT ACT CCA TGT 2508
 S P D S T S V C V Q G Q C V K A G C D R 703
 ACG CCA GAT TCC ACC TCT GTC TGT GTC CAA GCA CAG TGT GTC AAA AAT GCT GGT TGT GAT CGC 2568
 I I D S K K F D K C G V C G G N G S T 723
 ATC ATA GAC TCC AAA AAG AAG TTT GAT AAA TGT GAT GTC CGG GGA AAT GGA TCT ACT 2628
 C K R I S G S V T S A K P G Y H D I I T 743
 TGT AAA AAA ATA TCA CGA TCA GTT ACT AGT GCA AAA CCT CGA TAT CAT GAT ATC ATC ACA 2688
 I P T G A T N I E V K Q R N Q R G S R N 763
 ATT CCA ACT CGA CGC ACC AAC ATC GAA GTG AAA CAG CGG AAC CAG AGG GGA TCC AGG AAC 2748
 N G S F L A I K A A D G T Y I L N G D Y 783
 AAT GCC AGC TTT CTT GCC ATC AAA GCT CCT GAT GGC ACA TAT ATT CTT AAT GGT GAC TAC 2808
 T L S T L E Q D I M Y K G V V L R Y S G 803
 ACT TTG TCC ACC TTA GAG CAA GAC ATT ATG TAC AAA GGT GTC TTG AGG TAC AGC GCC 2868
 S S A A L P R I R S F S P L K E P L T I 823
 TCC TCT CGG CGA TTG GAA AGA ATT CGC AGC TTT AGC CCT CTC AAA GAG CCC TTG ACC ATC 2928
 Q V L T V G N A L R P K I K Y T Y F V K 843
 CAG GTT CTT ACT CTG CGC AAT GCC CTT CGA CCT AAA ATT AAA TAC ACC TAC TTC GTC AAG 2988
 K K K E S F N A I P T F S A W V I E E W 863
 AAG AAG AAG GAA TCT TTC AAT GCT ATC CCC ACT TTT TCA GCA TGG CTC ATT GAA GAG TCG 3048
 G E C S K S C E L G W Q R R L V E C R D 883
 GGC GAA TGT TCT AAG TCA TGT GAA TTG GGT TGG CAG AGA AGA CTG GTC GAA TGC CGA GAC 3108
 I N G Q P A S E C A K E V K P A S T R P 903
 ATT AAT GGA CAG CCT GCT TCC GAG TGT GCA AAG GAA CTG AAG CGA CCC AGC ACC ACC CCT 3168
 C A D H P C P Q W Q L G E W S S C S K T 923
 TGT GCA GAC CAT CCC TGC CCC CAG TGG CAG CTG GGG GAG TGG TCA TCA TGT TCT AAG ACC 3228
 C G K G Y K K R S L K C L S H D G G V L 943
 TGT GGG AAG GGT TAC AAA AAA AGA AGC TTG AAG TGT CTG TCC CAT GAT CGA GGG GTG TTA 3288
 S H E S C D P L K K P K H F I D F C T M 963
 TCT CAT GAC ACC TGT GAT CCT TTA AAG AAA CCT AAA CAT TTC ATA GAC TTT TCC ACA ATG 3348
 A E C S - 968
 GCA GAA TGC AGT TAA 3363
 GTGGTTTAAAGTCGTCTTACCTTGTAGGGCAAGGCAAAGTGAGGAAGGGCTGGTGTACGTTAGAAGAAGGCTGGAGCC 3442
 ATCCAGCGTATCTTGCAGTAAACCGTACGGCTGATCTGAGCTGGATTATGGGGCTAGATGAGAAAGGAGTTGAAT 3521
 CATCAGAGTAAACTGCCAGTTGCAAAATTGTAGGATAGTTAGTGAGGATTATTACCTCTGAGCAGTGATATAGCATA 3600
 ATAAAGCCCCGGCATTAATTATTTTCTTGTACATCTATTACAAGTTAGAAGAAAACAAAGCAATTGTCAAA 3679
 AAAAGTTAGAACTATTACAACCCCTGTTCTGGTACTTATCAAATCTTAGTATCATGGGGTTGGAAATTGAAAAGT 3758
 AGGAGAAAAGTGAGATTTTACTAAGACCTGTTTACTTACCTCACTAACAACTGGGGGAGAAAGGAGTACAAATAGGA 3837

TCTTGGACCAGCACTGTTATGCCAGCTGTGGTTTCAGAGAATGTTTACATTATTTCACGAGAATTAAACTCA 3916
GATTGTTCAACATGAGAGAACGCTCAGCAACGTGAAATACGCAATGGCTTCTCTTCCCTTTGGACCACTCTCA 3995
GTCCTTATTGTTGTAATTCAATTGAGGAAAAACAACTCCAGTATTATTCAGTGCATTAAGTCACAAATGGAAA 4074
AAARGCAGTGAACCATTACATGCTGTAAGCTAGAGGAGACACATGAGCTTAGTACCTCCAACTTCTTCCTTC 4153
TACCATGTAACCTGCTTCGGAAATAGGAGTAAAGAAGTAATTGGTGTCTTGAAATACGTACAAATCACAGG 4232
AGGATGAAACCCGGAAACAAAATGAGGTGTGAGAACAGGGTCCCACAGGTTGGGACATTGAGATCATTGCTTG 4311
TGGTGGGGAGGCTGCTGAGGGTAGCAGGTCATCTCCAGCAGCTGCTTCAACAGTCTTCTGGTAAATGTCAGTC 4390
AGCTCTCTGAGAATATGATTTCATATGTTAGTAAATAGTTACTTAAATACATGTACTTTATAAGT 4469
ATGGTTTGGGTGTTCTTCCAAGAAGGACTTAACTTGTGTTAAATGCCATAATAACATATTATTTTATAACATT 4548
ATTCTAAATGAAAAAAACTTTAAATTATATGCCCTTGTGGAACTGCAATAAAATAGAGTATTATACAATATATGT 4627
TACTAGAAATAAAAGAACACTTTTGGAAAAA
4676

Fig. 2 (1 of 2)

CACAAATCCGTACTCTGACCCATAGCACTCTTTTCTTCTTAAATTTCTCTCTGCTAACTGAGTACCTGCATGCTGTC 1744
 CCCAGGTCTTGTAAACACAACCTTTATGATTGAAATTAAAGCTACTTATTCTAGTTTATACCCCTAAACTACCT 1823
 TTTTGTTTCCATTCCTTAAATTGTATTTGTTTCCCTAGTGTAAATTCTCATGCTTTTATATCTCTTAAATAAGGTGTGG 1902
 TCTGTCTTCTGAAACAAAGTGTAGACTTCTGGAGTCATAATCTGGTGAACAAATTCTCTGTAGCTGTAAAGCAG 1981
 TCACTTAATCTTCTACCTCTTTTCTCTCCAAATTGAGATAATGATACTTAAACCTAGTTAGAAGAGGTAGTGTGA 2060
 ATATTAATTAGTTAACTACTCTCACTCTTGAACATGAACCTATGCTATGAGTGTCTTATTGCTAGCTGGCTG 2139
 AGACACTGAAGAAGTCATGAAACAAACCTACACACGTACCTTCATGTGATTCACTGCTTCTCTCTACCCAGTCATA 2218
 TTTCCACTGAAACAAACCTACACACNTACCTTCATGTGCTTCAGTGCCTTCTCTCTACCCAGTCATACTGAA 2297
 CAAACCTACGACATACTTCATGTGCTTCAGTGCCTTCTCTCTACCCAGTCATACTGCTTCTCTGTC 2376
 TGACATTTTGTGCTCTGTTCCATTAAACACTCTCTACTTTCTCTGCTGTGAGAAAGCTTACAGAAAGCTATTCTGAGCA 2455
 AGATGATGTAATGGAAAGGGTGTGGCATGGTGTCTGGAGACCTGGATTGGTGTCTGGTGTCTATCAATCACCGTCTG 2534
 TGTGAGGCTAGGCATTGGCTGCTGTAAGCTTAATGCTCTGTAAGGGTGGTTGTAAATTCTGATCTTCCCAC 2613
 CTCACTGATGTTGTGGGATCCACTGAGATAAGAAATACATGTAAGTGTGGTTGTAAATTAAAAAGTGTATAACTAA 2692
 GGGAAAGAAATTGAGGAATTAACTGCCATACGTTTGTGGCTTTCAAAATGTTGAAAACAAAAAAATGTTAAGAAA 2771
 TGGGTTCTTGCCTTAACTGACTCTCTCAAGTGTGAGACAGTGAAGTAAAATTGAGTGTGCACTAAACAAAATAAGATTCTG 2850
 AGGAAGTCTTATCTCTGCAGTGTGATATGGCCCGATGCTTCTGTGGCTAAACAGATGTAATGGGAGAAAATAAGCC 2929
 TAACTTGGTAAATCCAAACGCAAGGGAGATTGTGAATCTATAATAACTCTATAAGGTCTTATCTGTTCACTGATGCC 3008
 TCAGAGCTCTCTGCTTGTAGCTGGCAGCTGACGGTGTAGGATAGTTAGTTGGAAAATGGTACTTCATAATAACTACAC 3087
 AAGGAAGTCAGCCACTGTGTCTTGTAGGAATTGGACCTAAATAAATTGAGTGTGCTTCCAAACCTGAGAAATATG 3166
 CTTTGGAAAGTTAAATTAAATGGCTTTGCCACATACATAGATCTCTGAGTGTGAGTGTAAATTCCATGTGGATA 3245
 TCAGTTACCAAAACATTACAAAAAATTATGGCCRAAAATGACCAACGAAATTGTTACAATAGAAATTATCCAAATT 3324
 GATCTTTTATATTCTTCTACCAACACCTGGAAACAGACCAATAGACATTGGCTTTATAATAGGAATTGTATAAA 3403
 GCATTACTCTTTTCAATAAAATTGTTTTAAATTAAAAAGGAAAAAAGGGGGGGCG 3482
 C 3483

Fig. 2 (2 of 2)

5'CGAACCCGCGCTGGAGAACCTTTCAGGCGACAAACTACGGGGCGATTCTGATTTGGCTT 73

M	G	L	W	G	Q	S	V	P	T	A	S	S	13								
ATG	GGA	CTT	TGG	GGA	CAA	AGC	GTC	CGG	ACC	GCC	TGG	AGC	142								
A	R	A	G	R	Y	P	G	A	R	T	A	S	G	T	R	P	W	L	L	33	
CTT	CGA	GCA	GGG	CGG	TAT	CCA	GGG	GCC	AGG	ACA	GGG	TGG	GGG	ACC	AGA	CCA	TGG	GTC	CTG	CTG	152
S	E	R	I	I	X	F	V	V	F	I	V	A	V	L	L	P	V	R	V	53	
GAC	TCC	AAA	ATG	ATC	CTT	AAG	TTC	GTC	GTC	TTC	ATC	GTC	GGG	GTT	CTG	CTG	CGG	GTC	CGG	GTT	162
D	S	A	T	I	P	R	Q	D	E	V	P	Q	Q	T	V	A	P	Q	Q	73	
GAC	TTC	GGG	ACC	ATC	CCC	CGG	CAG	GAC	GAA	GTT	CCC	CAG	CAG	ACA	GTC	GCC	CCA	CAG	CAA	172	
Q	R	R	S	L	K	E	E	C	P	A	G	S	H	R	S	E	Y	T	93		
CGG	AGG	CCC	ACC	CTG	AAG	GAG	GAG	GAG	TGT	CCA	GCA	GGG	TCT	CAT	AGA	TCA	GAA	TAT	ACT	182	
S	A	C	N	P	C	T	E	G	V	D	Y	T	I	A	S	N	N	L	P	113	
CGA	GCG	TGT	AAC	CCC	TGC	ACA	GAG	GAG	GGT	GTC	GAT	TAC	ACC	ATT	GCT	TCC	AAC	AAT	TTC	CTT	192
S	S	L	L	S	T	V	C	K	S	G	Q	T	N	K	S	S	C	T	T	133	
TCT	TGC	CTG	CTA	TGT	ACA	GTT	TGT	AAA	TCA	GCT	CAA	ACA	AAT	AAA	AGT	TCC	TGT	ACC	ACC	182	
T	R	C	T	V	C	Q	C	E	K	G	S	F	Q	D	K	N	S	P	E	153	
ACC	AGA	GAC	ACC	TTC	TGT	CAG	TGT	GAA	AAA	GGG	AGC	TTC	CAG	GAT	AAA	AAC	TCC	CCT	GAG	562	
M	C	R	T	C	R	T	G	C	P	R	G	M	V	K	V	S	N	C	T	173	
ATG	TCC	GGG	ACG	TGT	AGA	ACA	GGG	TGT	CCC	AGA	GGG	ATG	GTC	AAG	GTC	AGT	AAT	TGT	ACG	522	
P	R	S	D	I	K	C	K	N	E	S	A	A	S	S	T	G	K	T	P	193	
CCC	CGG	AGT	GAC	ATC	AAG	TGC	AAA	AAT	GAA	TCA	GCT	CCC	AGT	TCC	ACT	GGG	AAA	ACC	CAA	682	
A	A	E	E	T	V	T	T	I	L	G	M	L	A	S	P	Y	H	Y	L	213	
GCA	GCG	GAG	GAG	ACA	GTC	ACC	ACC	ATC	CTG	GGG	ATG	CTT	GCC	TCT	CCC	TAT	CAC	TAC	CTT	742	
I	I	I	V	V	L	V	I	I	L	A	V	V	V	V	G	F	S	C	R	133	
ATC	ATT	ATA	GTC	CTG	CTT	TTA	GTC	ATC	ATT	TTA	GCT	CTG	CTT	CTG	GTT	GGC	TTT	TCA	TGT	GGG	392
S	S	F	I	S	Y	L	K	G	I	C	S	G	G	G	G	G	P	E	R	153	
AAG	AAA	TTC	ATT	TCT	CTC	AAA	GCG	ATC	TCC	TCA	GCT	GGT	GGT	GGG	GGG	GGG	GGT	CCC	GAA	CCT	362
V	H	R	V	L	F	R	R	R	S	C	P	S	R	V	P	G	A	E	D	273	
GTC	CAC	AGA	GTC	CTT	TTC	CGG	CGG	CGT	TCA	TGT	CCT	TCA	CGA	GTT	CCT	GGG	GGG	GAG	GAC	922	
N	A	R	N	E	T	L	S	N	R	Y	L	Q	P	T	Q	V	S	E	Q	293	
AAT	GCC	GCG	AAC	GAG	ACC	CTG	AGT	AAC	AGA	TAC	TTG	CAG	CCC	ACC	CAG	GTC	TCT	GAG	CAG	982	
E	I	Q	G	Q	E	L	A	E	L	T	G	V	T	V	E	S	P	E	E	313	
GAA	ATC	CAA	GGT	CAG	GAG	CTG	GCA	GAG	CTA	ACA	GGT	GTC	ACT	GTA	GAG	TGG	CCA	GAG	GAG	1042	
P	Q	R	L	L	E	Q	A	E	A	E	G	C	Q	R	R	R	R	L	L	V	333
CCA	CAG	CCT	CTG	CTG	GAA	CAG	GCA	GAA	GCT	GAA	GGG	TGT	CAG	AGG	AGG	AGG	CTG	CTG	GTT	1162	
P	V	N	D	A	D	S	A	D	I	S	T	L	L	D	A	S	A	T	L	353	
CCA	GTC	AAT	GAC	GCT	GAC	TCC	GCT	GAC	ATC	AGC	ACC	TTG	CTG	GAT	GCC	TGG	GCA	ACA	CTG	1162	
S	E	G	H	A	K	E	T	I	Q	D	Q	L	V	G	S	E	K	L	F	373	
GAA	GAA	GGA	CAT	GCA	AAG	GAA	ACA	ATT	CAG	GAC	CRA	CTG	GTC	GCG	TCC	GAA	AAG	CTG	TTT	1222	

? E E D E A G S A S C L * 387
 TAT GAA GAA GAT GAA GCA GGC TCT GCT ACG TCC TGC CTG TGA 1264
 AAGATCTTTTACGGAAACCGAGCTTCCCTGATTACCTTTCTCTACAAAGGGAGCAGCTGGAGAAACAGTCC 1343
 ACTACTTACCCATGCCCTACAAACTCTACTATOCATATGGGGCAGCTTACCAATGGTCCAGACTTTGTCACGC 1422
 ACTTGGAGTAATTTTATGAAATACTGCGTGTGATAAGCAAACGGGAGAAATTATATCAGATTCCTGGCTGCATAGTT 1501
 ATACGATTGTCTTAAAGGGTCCTTTAGGGCAGCTGCGTGCATGCCCTAAATCCCAGCAGCTTGAAGGCTGAG 1580
 SCAGGTCGATTCTTGGCTCCGGAGTTGAGACCGAGCTCTAACACAGTGAACACTCCATCTCAATTAAAAAGAAA 1659
 AAAAGTGTCTTAGGATGTCACTTTGCAGTTCTCATGAGACAAGTCCTTTCTGCTTCTTATATTGCAAGC 1738
 TCCATCTTACTGGTGTGCAATTATGACATCTAACTACAGATGCCAACAGCCACAATGCTTGCCTTATAATT 1817
 TTAACTTTAGAACGGGATTATCTGTTATTACCTGTATTTCAGTTTCGGATAATTGTACTAAATGATGAGATTATCA 1896
 AGACGTAGCCCTATGCTAAGTCATGAGCAATGGACTTACGGGGGTCGACTTAGAGTTTGAGCTTAAAGATAAGATT 1975
 ATGGGGCTTACCCCCACCTTATTAGAGAACATTATATTGCTTACTACTGTAGGCTGTACATCTCTTCCGATT 2054
 TTATATAATGAGTAAACATGGAAAAACTTTAGGAATGCACTTATTAGGCTGTTACATGGGTTGCCTGGATACAAAT 2133
 CAGCACTAAAAATGACTAAAAATATAACTAGTGACGGAGGGAGAAATCTCCCTCTGGGGAGGCACCTACTGCAATC 2212
 CAGTCTTCTTCTTCTGGGCTTGAGACTGGACCAGGGTTGA/CGCTGGCACCTCTCAAGGGCAGCTTGTCTTACTTG 2291
 TTAAATTAGGGTATATAGCCATATTATATAAAATAATTATTTATTATAAGTAGATGTTACATATG 2370
 CCCAGGATTTGAAGACCTGGTATCTTGGGAGCCATGTGTCCTGGTTGTCGTCGGACAGTCATGGGACTGCA 2449
 CTTCCGACTTGTCCACAGCAGATGAGGACAGTGAGAATTAGTTAGATCCGAGACTTCGAAGAGCTCTCTTCAAGCG 2528
 CCATTACAGTTGAACGTTAGTGAATCTTGGCCTCATTTGGCTCAGGCAGAGCAGGTGTTATCTGCCCCGGCATCT 2607
 CCATGGCATCAGAGGGAGAGTGGACGGCTTGGGATCGTGTGAAATGGTTGGGACTCAGGCATGGATGGGCC 2686
 TTTCTCTTCTGGCTGTGAACTGAGTCTTGGATGCTTTAGGGAGAGATTCTGAGCTGGTTTAGGGTA 2765
 TAGATTCTTCTTGGAGGACTTGGCCCTCTTAAGCATCTGACTCATCTGAGAGATATCAATTCTTAAACACTGTA 2844
 CAACAGGATCTAAATGGCTGACACATTGTCCTGTGTCAGTTCATTATTTATTTAAACCTCAGTAATCGTT 2923
 TAGCTTCTTCCAGCAAACCTCTTCCACAGTAGGCCAGTGGTAGGATAAAATACGGATATAGTCATTCTAGGGT 3002
 TTCACTTCCATCTCAAGGATTGTGTTGCTGGACTTGGAGTTAGGAGGGAGGGCTTCCGGCTTCCGACTTCCTCTCC 3081
 CTTCGACATTCAGATTGTGTCCTGGAGTTAGGAGGGAGGGCTTCCGGCTTCCGACTTCCTCTCC 3160
 CACTTCCATCTGGCTCCACACCTTGTCCCCCTGCACCTCTGGATGACACAGGGTGGCTGCTGGCTCTAGTCCTGCT 3239
 TTCTGGGCTTCTGTGCAAGGAGACTTGGCTCTCAAGCTCAGAGAGGCCAGTCGGTCCAGCTCTTGTGCTTCC 3318
 TGTGAGGGCTTCTTGAAGATGCATCTAGACTACCAAGCTTATCACTGTTAAGCTTATTCCTTACATAAGCTTCT 3397
 GACACATGAAATTGTGGGTTTGGCTTGGTGAATTGTTAGGTTTGTCTTATACCCGGCCAAATAGCACA 3476
 TAAACACCTGGTTATATGAAATACTCATATGTTATGACCAAAATAATATGAAACCTCATATTAAAAAAA 3555
 AAAAGGGCGGCC 3569

GCCTTTAGGGCAGAGATTCTGAGCTGCGTTTCTAGGGTACAGATTCTGTTT
GAGGAGCTGGGAACTCTGTAAGCATCTGACTCATCTCAGAGATAT
CAATTCTTAAACACTGTGACAAACAGGATCTAAATGGCTGACACATTTGT
CTTGTGTCAGTTTCACTTATTCTTATTCTTAAAAACCTCAGTAATGTTTA
GCTTCTTTCAGCAAACCTCTTCACTACAGTAGGCTGAGCTGTTGGTAGGATA
AATTAAAGGATATAGTCATTCTAGGGGTTTCAGTCTTTTCACTCTCAAGGC
ATTGTTGTTTGTGTTGGGACTGGTTGGCTGGGACAAAGTTAGAACTG
CTGAAAGTTGGCACATTAGATTGTTGTCATGGAGTTTAGGAGGGG
ATGGCTTCTTGGGCTTGGCACATTCACTCTCCTGACTCTGGG
TOOCACACCTTGTGTTGGGACTCTGACTCTCTGGATGACACAGGGGCTGCTGCTGCT
CTAGTCCTTGCCTTGTGCTGGGOCCTCTGTCAGGAGACTTGGCTCTCAA
GCTCAGAGAGAGOCAGTGGTDOAGCTCTTGTGCTTCTCAGAGG
CTTCTCTGAAGATGCATCTAGACTAACAGGCTTATCAGTGTAAAGCTT
ATTCTTAAACATAAGCTTCTGACAAACATGAAATTGTTGGGGTTTGTG
GOGTTGGTTGATTGTTAGGTTTGCCTTATAACGGGCGAACATAGCAC
ATAACAACTGGTTATATATGAAATACTCATATGTTTATGACCAAAATAAA
TATGAAACCTCATTTAAAAAAAAAAAAAGGGGGGGG

Fig. 4

36.2% identity in 176 aa overlap

Fig. 5

V R N K T L P W S P C S A V Y L T E L	19
GC GTC CGG AAC AAG ACG CTG CCC TGG TCT CCC TGC AGT GCT GTC TAC CTC AGC GAG CTC	59
I D D G H G D C L L D D G H S T L Y E L	39
CTG GAT GAT GGT CAC GCA GAC TCC CTC CTG GAT GAT GGC CAC AGC ACG CTC TAT GAG CTC	119
C Q Q C X Q I F G P D F R H C P N T S V	59
GAC CAG CAG TCC AAG CAG ATC TTT GGG CCT GAT TTC CCA CAC TCC CCC AAC ACC TCT GTG	179
E D I C V Q L W C R H R D S D E P I C H	79
GAG GAC ATC TGT GTC CAG CTC TGG TCC CTC CAT CGG GAT AGT GAT GAG CCC ATT TGC CAC	239
T K N G S L L W A-D G T P C G P G H L C	99
ACA AAG AAT GGC AGC TTG CTC TGG GCT GAT GGT ACG CCC TGT GGC CCT GGG CAC CTC TGC	299
L D G S C V L R E E V E N P K A V V D G	119
CTG GAT GGT AGC TGT GTG CTC CGG GAG GAA GTC GAG AAT CCC AAG CCT GCT GTC GTC GAT GGA	359
D W G P W G P W G Q C S R T C G G G I Q	139
GAC TGG GGT CCC TGG GGA CCC TGG GGA CAA TGT TCT CGC ACC TGT GGT GGA GGG ATA CAG	419
F S N R E C D N P A P Q N G G R F C L G	159
TTT TCG AAC CCT GAG TGT GAT AAT CCA GCA CCT CAG AAT GGA GGA AGA TTT TGC CTG GGA	479
E R V K Y Q S C K T E E C P P N G K S F	179
GAG AGA GTC AAG TAC CAA TCT TCC AAG ACA GAG GAA TGT CCA CCA AAT GGA AAA AGC TTC	539
R E Q Q C E K Y N A Y N H T D L D G N F	199
AGG GAG CAG CAG TGT GAA AAA TAT AAT GGC TAC AAC CAC ACG GAC CTG GAT GGG AAT TTC	599
L Q W V P K Y S G V S P R D R C K L F C	219
CTT CAG TGG GTC CCC AAA TAC TCA GGA GTG TCC CCC CGA GAC CGA TGC AAA CTG TTT TGC	659
R A R G R S E F K V F E T K V I D G T L	239
AGA GCA CCT CGG AGG AGT GAG TTC AAA GTG TTT GAA ACT AAG GTG ATC GAT GGC ACT CTG	719
C G P D T L A I C V R G Q C V K A G C D	259
TCC CGA CGG GAT ACT CTG GCC ATC TGT GTG CGG GGA CAG TGC GTT AAG GCT GGC TGT GAC	779
H V V N S P K K L D K C G V C G G K G T	279
CAT GTG GTG AAC TCA CCT AAG AAG CTG GAC AAG TGT GGG CTG TGT GGG GGC AAA GGC ACT	839
A C R K V S G S F T P F S Y G Y N D I V	299
CCC TGT AGG AAG GTC TCA GGT TCT TTC ACC CCC TTC AGT TAT GGC TAC AAT GAC ATT GTC	899
T I P A G A T N I D V K Q R S H P G V Q	319
ACC ATC CCA GCT GGT GCC ACA AAT ATT GAT GTG AAA CAA CGG AGC CAC CCA GGG GTC CAG	959
N D G S Y L A L K T A N G Q Y L L N G N	339
AAT GAC CGC AGC TAC CTG GCA CTG AAG ACA GCC AAT GGG CAG TAC CTG CTC AAT GGT AAC	1019
S A I S A I E Q D I L M K G T I L K Y S	359
CTA GCC ATC TCT GCC ATA GAG CAA GAC ATC TTG ATG AAG GGG ACC ATC CTA AAG TAC AGT	1079
G S M A T L E R L Q S F Q A L P E P L T	379
GCT TCC ATG GCC ACC CTG GAG CGG CTG CAG AGC TTC CAA GCC CTC CCT GAG CCT CTT ACA	1139

Fig. 6 (1 of 2)

V Q L L T V S G E V F P P K V K Y T F F 399
 GTA CAG CTC CTG ACT GTG TCT GGT GAG GTC TTC CCT CCA AAA GTC AAA TAT ACC TTC TTC 1199
 V P N D T D F N V Q S S K E R A S T N I 419
 GTC CCC AAT GAC ACG GAC TTC AAC GTG CAG AGT ACC AAA GAA AGA GCA ACC ACC AAC ATC 1259
 I Q S L P Y A E W V L G D W S E C P S T 439
 ATT CAG TCC TTG CCC TAT GCA GAG TGG GTC CTG CGG GAC TGG TCT GAA TGT CCA AGC ACA 1319
 C G G G W Q R R T V E C R D P S G Q A S 459
 TCT GGA GGT GGC TGG CAG CGG CGG ACT GTG GAA TCC AGG GAC CCC TCA GGT CAG GCC TCT 1379
 D T C D E A L K P- E D A K P C G S Q P C 479
 GAC ACC TGT GAT GAG GCT CTG AAA CCT GAG GAT GCC AAG CCC TGT GGA AGC CAG CCA TGT 1439
 L L * 482
 CTC CTC TGA 1448
 TCCCTTTGGACATGCTAAGGCTATGGATTTGGCTACTGGCTACAGACAAAGGTCTCTCTGAGTCACATA 1527
 TATCAAGATGCGATGGCCCTTCCGGCTTAACTACAAACCTTGGTACCCCTTAATTCATAGGAGAGAG 1506
 AAGAGGAAGTAAAGGTAACAGACTGTAAGTTGACTGTCAGTGGACTGGACCTTCTTATGACCAAGAAGATGGATA 1685
 GGTAAAACGTTAAAGCTTAAAGCTTATGCTCAGGAGATTCTAGAACCCGCTCTTCTGCAAAAGGACTAGAAAGGTT 1764
 AAATGAGAAAGAGAGTTTTTTCTTCTGTTCTCCATAATCTAACTCTACCTCACAGGGGGAGGAACTTGGTGTAT 1843
 AAGCCCTGGTGTAGTGGTAGTCAGGCACTCTCCATAGATATCTTGGACCCATCTCAGAAAATGCCATGGCTGT 1922
 TTTCAGTATTAAAACCTCTGTTGCTAAAGGTGGTGGTGTCCATCACAGGTTATAGAAAGGCACTTGTCTCAGGCT 2001
 GCTCTCTCTGGGGGGGAGCCCTTCTAGTATTCTGCAAAATCTTCTGACTAAAGTGTGATCTTACACCAAAAAA 2080
 AAAAAAAAAAAAAAAAAAGGGGGGGG 2114

Fig. 6 (2 of 2)

>fhxe29c9F, 1448 bases, 3233 checksum.

GTGGAGGGAGGGTGGGAGCTGGCAGAGATGCAAGCTGCAAGCTGCAA
AATGGGGCAGACTGTGACAGTGTGACTGGAAAGTCACTGTGCCCCAGG
ATTCAGGAAATTGACTGTCTAACCCTGAACTCTGGAAACTATGGGA
TAAACTGTTOCTCTGCTGTGGCTGAAAAATGATGCAAGTCTGCTCTCT
GTGGAGGGCTTGACTTGCAAGGCAGGCTGGCAAGGGTGGACTGCTC
CATCAGATGTOOCAGTGGCACATGGGGCTTGCTGTAACCTAACATGAC
AGTGCCTCAAGGGGGAGGCTGCAACAACTGGAGGGAGCTGCAAGTGT
GCACTGGATGGGGGGAGAAATGCGAACCTGCTGCAAGGGATGGCAC
GTAAGGGCTGAACTGTGCTGAGGCTGCGACTGCAAGOCAGAGATGGCT
GCGAACCTAACAGGGGCAATTGCGCTGCGTGGGGAGCTGCAAGGTTG
CACTGTGACAGGGTGTGCTGAGGGAGGCTGGGGGGGGCAACTGCTGCT
GCGAGCTGCTGCAACAGGGCTGGGGGGGGGGGGGGGGGGGGGGGGGG
CCTGGTTTATGGGCATGGCTGCAAGGGAGACATGGGGGGGGGGGGGGGG
CAGCAGGG
GCTCACAGGG
AAAAACTGTGCAAGGAATTGTACTGCAACCAACAAAGGGAACTGTAACCC
CATGACAGATCTTGTCAGTGTGTTAACCGGGTTGGGATGGCAGTGACTGCT
CTCAACCATGTCACGCTGGGCACTGGGGGGGGGGGGGGGGGGGGGGGG
AACTGOCATAATGGAGCTCTGCAAGGGGGGGGGGGGGGGGGGGGGGGGG
CACTGGCTGGGACAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
TTTATGGAAAAGATTGTGCACTGATATGCGAACATGCAATGTCAAAAGGG
TGCGACACATTTCTGGGCAAGTGTGACTTGGGGGGGGGGGGGGGGGGGG
GGACTGTGAGGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
AGATATGTGATTGTGCAACAACTGCAACGCTGGGGGGGGGGGGGGGGGG
TGTTACTGCAAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
TATCATAGTTGGAAATCTGAAACAGCTTAAGGCGAACATGGCTGCGTCTOC
CTGCTGATTCTGCAACAAATGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
CTAGTTGTTCTCTGCACTGGCATTGTTCAATTATTTATAGACACACAGC

Fig. 7

>fthxe29c9R, 1578 bases, 5059 checksum.

NAGOCACAGGAATGTTCTATGAAAGTGAACCTAACAGTGAGTGTGTT
CCAAGGAGTATTCAAGCAATAATGGGCGTCNTCCAGGATOCATATGA
OCTCCAAAGAACAGTCACATOCCTGTCAATTATGACAGTGCTGOCAGTOC
GAGACAGTTCACTCTCAGCAAGAGGACAGTGGAGGTAGCAGCAGC
AACAGCAGCAGCAGCAGTGAATGACAOCAAAGGAOCCTGGTAGOCAGT
GGAAOCCTTCAGAACAGTGTGTTGGTTCTCTOCATOCCTCAATTTC
CACTTCATGTGAATGTTAGTCATTGGTGGCAATTGGACATGAA
CCAGAAAGCTGAAAGCTGAGGCTGACAOGGACTGTAGGTGCTTTGTC
AGGTGGATTGAGGAGTTAGAGATGTGATTGOCATTGCTGTTAGTTT
AGAACTATAACCGTGAAGCATGACTTATTGTAAGATGTGGCTGAAAGCA
TGAACCTGCAGAACACTCTGGAGACGAGGTGCACTGGACATTGGAT
TGTGCTGAAAAATTAAATTGAATATTCTCTCTCATTTGATCAT
ACAGCTCTAACCTAGGATTGTACAGTTAACATAAAATTACTTCATGAA
GTGGGAATCACTGAACATGAGAACAGAACATATTGTTAACCTGCT
ATTCTTAACCTTATTCAACTGGACTCAGAATTGTTAGGGATAATATGAATG
CAGGAGGAAACATTCTGTCAGGCGGTATGACTGGACAGACTTGAATATA
CTCTAAAAGTGGACAGAAAATTACGAAAATTAGATTAGGAAGTATTGCA
GAGAAAATACAAATTAGAATTATTAGAAATAGTGGAAAGTATTGCA
AAGTCATAACACAAATGTGCCAGGCAGAGGTGTTCTCTGTTGACTC
TCAACCAACTTCAGATCTATGACATTCTGATCACTGGCTOCATCATA
CATATTCAACACTTGAGATTCTACATACATCAATAGTTATTCTATAAATA
TAGAAATGAAATAATTATTATTGACAGACTGGATGGAATGAGTGTGTA
ATGATTGATAAAGGTTGAAATTAAATGCAAGATGACGCTTAAGTTCT
GTAACATTAGTAATACATGCTGTAATATAGAATTAGTGGAACATTG
ATTAAATCTTCTGACTGAAAGTGAATGAAATTGTCATTAGAGAACTATGCTTAAGC
TGGCTTTGCAATTGCTAATGTGACATGTACCCAACCTTCTATTGAGAA
TATTGCAATTGCTATTGTCATTGAGAACTATGCTTAAGC
TAATGAAAAAATTATGTTGTTATTGTCATTGAGAACTATGCTTAAGC
CTTGTGATAAAAATGCGTTGTTCTTTCTTTGGAGGGACAAGA
TGAAAATATAATTGAAATTGATTAATTGTCGTTACTAAAATAGTA
TAGTAAAAAAAAAGGGGGCG

Fig. 8

TANGO 71/ADAMTS-1 Comparison (90% Protein Sequence Identity)

251 DQSMADFHGSGLAKYLLTLFSVAARFYKRPSTIRNISLIVVVKILVITYEQ 300
 ||||.::||
 1.....TRPILVIRHDEQ 11

301 KGPEVTSNAALTIRNFCSWQRQHNSPSDIRDPEHYDIAILFTRQDLCGSHT 350
 |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
 12 KGPEVTSNAALTIRNFCSWQRQHNSPSDIRDPEHYDIAILFTRQDLCGSQT 61

351 CDTLGMAQVGTVCOPSRSCSVIEDDGLQAAFTTAHELGHVFNMPHDIAK 400
 |||||:|||||:
 62 CDTLGMAQVGTVCOPSRSCSVIEDDGLQAAFTTAHELGHVFNMPHDIAKQ 111

401 CASLNGVSGDASHLMAASMLSSLDRHSQWPWSPCSAYMITSFLDNGHGECLMDK 450
 |||||:|||||:
 112 CASLNGVQNQDSHMMASMLSSLDRHSQWPWSPCSAYMITSFLDNGHGECLMDK 161

451 PQNPINLPSDLPGTFLYDANROCOFTFGEESKHCDAASCTTLNCIGTSG 500
 |||||:|||||:
 162 PQNPINLPGDOLPGTSYDANROCOFTFGEEDSKHCDAASCTTLNCIGTSG 211

501 GLLVQQTQHFPWADGTSCEGKNCVSGKCVNKTDMKHFATPVHGSWGPNG 550
 |:|||||:|||||:
 212 GVLVQQTQHFPWADGTSCEGKNCVNGKCVNKTDRHFDTPFHGSWGMNG 261

551 PWGDCSRICGGGVQYIMRECINPVPKNGGKCEGKRVRYRSCNIEDCPDN 600
 |||||:|||||:
 262 PWGDCSRICGGGVQYIMRECINPVPKNGGKCEGKRVRYRSCNIEDCPDN 311

601 NGKTFREEQCEAHNEFSKASFGNEPTVEWTPKAGVSPKDRCKLTCERKG 650
 |||||:|||||:
 312 NGKTFREEQCEAHNEFSKASFGSGPAVEWIPKAGVSPKDRCKLICQAKG 361

651 IGYFFVLUQPKVVDGTPCSPDSTSVCVQGQCVKAGCDRIIDSKKFDKCGV 700
 |||||:|||||:
 362 IGYFFVLUQPKVVDGTPCSPDSTSVCVQGQCVKAGCDRIIDSKKFDKCGV 411

701 CGQNGSTCKRMSGIVTSTRPGYHDIVTIPIAGATNIEVKHRNQRGSRNNGS 750
 |||||:|||||:
 412 CGQNGSTCKKISGSVTSAKPGYHDIIITIPIGATNIEVKQRNQRGSRNNGS 461

751 FLAIRAADGTYILNGNFTLSTLEQQLTYKGTIVLRYSGSSAALERIIRSFSP 800
 |||||:|||||:
 462 FLAIRAADGTYILNGNFTLSTLEQQLTYKGVVLRYSGSSAALERIIRSFSP 511

801 LKEPLTIQVLVGNALRPKIKFTYFMMKQKTESFNAIPTFSEWVIEEWGEC 850
 |||||:|||||:
 512 LKEPLTIQVLVGNALRPKIKFTYFVKKKRESFNAIPTPSAWVIEEWGE. 560

901 WSPCSKTCGAGYKKRTLRCVSHDGGVLSNESCDPLKGPKHYIDFCILTQC 950
 |||||:|||||:
 561 ...CSKTCGAGYKKRTLRCVSHDGGVLSNESCDPLKGPKHYIDFCILTQC 607

951 S* 951
 |
 608 S* 609

Fig. 9

COMPARISON OF TANGO 73 AND TMVCF
(SIROTKIN ET AL. GENOMICS 1997, 42:245-251)

PERCENT SIMILARITY: 67.619 PERCENT IDENTITY: 46.190

TMVCF.PEP X T73PRO JULY 29, 1997 16:32 ..

Fig. 10

Comparison of TANGO 73 and RVP1

Percent Similarity: 68.750 Percent Identity: 48.077

t73pro x RVP1.pep July 29, 1997 17:38 ..

1 MANAGLQLLGFI LAFLGWIGAIVSTALPQWRIYSYAGDNIVTAQAMYEGL 50
.. :|:: | .|||:..||:||| :||: .. |..|:||| :|||
1 .MSMSLEITGTS LAVLGWLC TIVCCALPMWRVSAFIGSSIITAQITWEGL 49

51 WMSCVSQSTGQIQC KVFD SLLNLSSTLQATRALMVGILLGVIAIFVATV 100
||.|| | | | |:| :| :| . .||.|||:|||:|||:| :| |
50 WMNCV.QSTGQM QCKMYD SLLALPQDLQ AARALIVV SILLA FGLLVALV 98

101 GMKCMK CLEDDEVQKMRM A VIGGA IFLLAGL AILVATAWYGNRIVQE FYD 150
| .| .|:| :| ..| :| :| :| .| :| :| ..| :| | :| :|:
99 GAQCTNCV.QDETAKAKITIVAGVLFLLA AVLTLYPVWSANTII RD FYN 147

151 PMTPVNARYEFGQALFTGWA AASLC L LGGALLCCSCPRKTTSYPTPRPYP 200
| :| ..| :| :| :| .| | | | | | | | | | :| ..| | .| .|
148 PLVPEAQKREMG TGLYVGWAAA ALQLGGALLCCSCPPREKYAPTKILYS 197

201 KP...APSSGKD YV..... 211
| :| :| ..| :|
198 APRSTGPGTGTAYDRKTTSERPGARTPHHHHYQPSMYPTRPACSLASE 247

Fig. 11

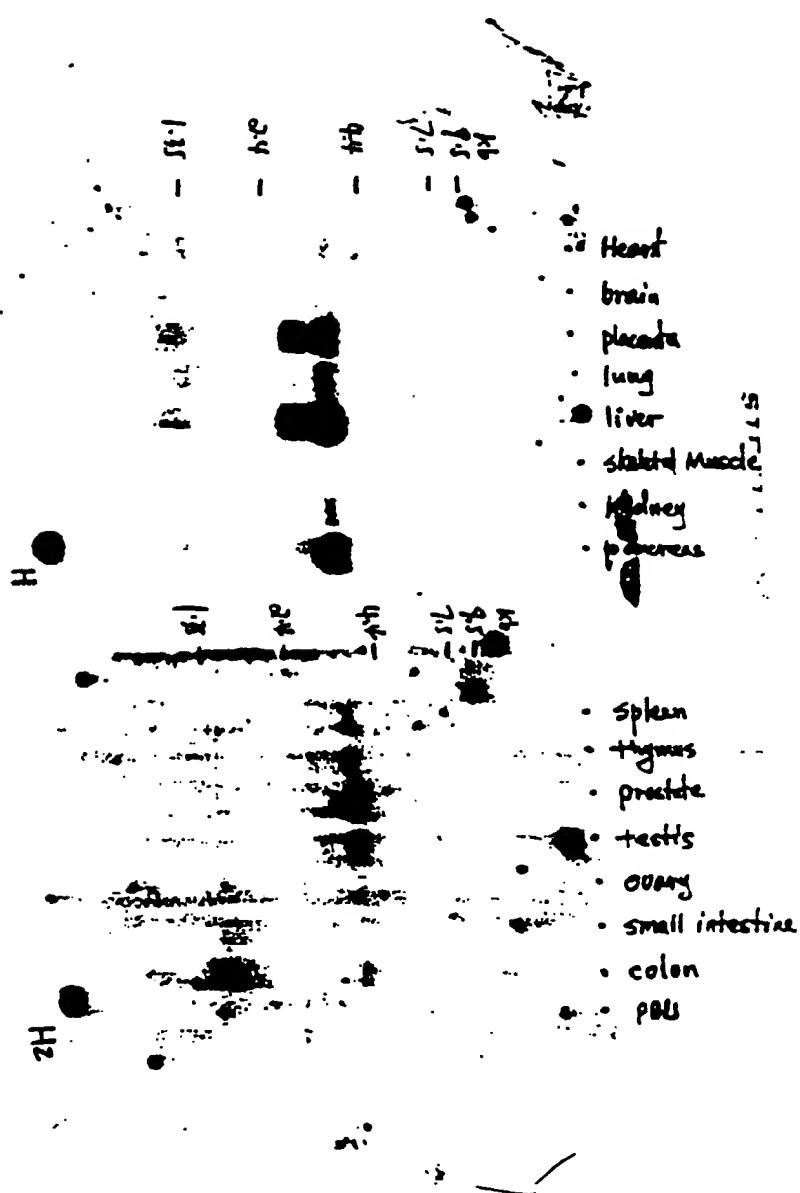


FIG. 12

1 2 3 4 5 6

- Heart
- Brain
- placenta
- lung
- liver
- skeletal muscle
- kidney
- pancreas

4

1 2 3 4 5

- spleen
- Thymus
- prostate
- testis
- ovary
- Small intestine
- colon
- PBL

5H

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16502

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/12, 15/16, 15/18, 15/63; C07K 14/46, 14/47, 14/475
US CL :530/350; 435/69.1, 71.1, 71.2, 325, 471, 252.3, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/69.1, 71.1, 71.2, 325, 471, 252.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

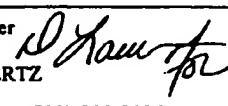
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/01896 A1 (HUMAN GENOME SCIENCES, INC.) 25 January 1996, see entire document.	1-10, 12
A	BRADHAM et al. Connective Tissue Growth Factor: a Cysteine-rich Mitogen Secreted by Human Vascular Endothelial Cells Is Related to the SRC-induced Immediate Early Gene Product CEF-10. The Journal of Cell Biology. September 1991, Vol. 114, No. 6, pages 1285-1294, see entire document.	1-10, 12

<input checked="" type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
A	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"Z"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
14 OCTOBER 1998	12 NOV 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  PREMA MERTZ Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/16502

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASON et al. Dorsal Midline Fate In Drosophila Embryos Requires Twisted Gastrulation, A gene Encoding A Secreted Protein Related To Human Connective Tissue Growth Factor. Gene & Development. 1994, Vol. 8, pages 1489-1501, see entire document.	1-10, 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/16502

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, MEDLINE, CAPLUS, EMBASE, BIOSIS
search terms: Tango-71, Tango-73, Tango-76, Tango-74, Tango-83, nucleic acid, DNA, polypeptide, protein, recombinant, cloning, production

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups invention which are so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I. Claims 1-10 and 12, drawn to Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, a vector, a host cell, and a method for producing Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides.

Group II. Claim 11, drawn to antibodies which bind Tango-71, Tango-73, Tango-74, Tango-76, and Tango-83 polypeptides.

Group III. Claims 13-15, drawn to a method for detecting Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides in a sample.

Group IV. Claims 16-18, drawn to a method for detecting nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, & Tango-76 and Tango-83 polypeptides in a sample.

Group V. Claims 19-22, drawn to a method for identifying a compound which binds to Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, nucleic acid molecules encoding Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides, a vector, a host cell, and a method for producing Tango-71, Tango-73, Tango-74, Tango-76 and Tango-83 polypeptides. Further pursuant to 37 C.F.R. § 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.